The University of Chicago

STUDIES ON THE REPRODUCTIVE SYSTEM OF THE MALE GUINEA PIG

I. POSTNATAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM IN MALE GUINEA PIGS AND ITS RELATION TO TESTIS HORMONE SECRETION

II. THE EFFECTS OF CASTRATION UPON THE REPRODUCTIVE ORGANS OF THE ADULT MALE GUINEA PIG

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
1942

By
EVERETT DUANE SAYLES

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E. DUANE SAYLES

POSTNATAL DEVELOPMENT OF REPRODUCTIVE SYSTEM IN MALE GUINEA PIGS AND ITS RELATION TO TESTIS HORMONE SECRETION[1]

(One figure)

E. DUANE SAYLES
Department of Zoölogy, University of Chicago, and
Virginia Junior College

A STUDY of the effects of castration on the guinea pig introduced so many questions that had a bearing upon the differentiation of the reproductive system from the postnatal to the mature functional state that it was deemed advisable to investigate the prepuberal development of this system. Such a study in the rat has been carried on by Price (1936), and for the rat testis by Moore (1936). Some of the data obtained from such a study on the guinea pig are reported here, together with some of the effects of prepuberal castration, which should aid in interpreting normal reproductive development. The effects of castration in mature males will be reported elsewhere.

This paper will concern itself with the general growth and histology of the testis and accessory organs of reproduction and with the functional differentiation of the testes, prostate gland, and seminal vesicles; Cowper's gland in the guinea pig has already been studied by Heller (1932). The main points to be stressed are the time at which germ-cell differentiation occurs in the testis and the discharge of spermatozoa in the ejaculate, and the correlation of these events with evident signs of testis hormone secretion, the indications for which should be registered by changes in the accessory organs of reproduction. Effects of castration in the prepuberal males has been of some aid in the principal interpretations of some of these events.

MATERIAL AND METHODS

One hundred and eighty-five animals were utilized in this investigation: they were secured from active breeding colonies at the University of Chicago; for many of the younger animals the writer is indebted to Dr. Sewall Wright. Tissues for fixation were removed from animals immediately after death. The removal of the urethra and annexed parts permitted an adequate separation of the anterior lobe of the prostate from the middle and posterior lobes, which were not separated from each other for purposes of weight records. A torsion balance and chainomatic balance were employed for fresh gland weights. Eight different fluids for fixation and several stains were utilized in preliminary searches for specific cell organs or inclusions that might prove adequate as diagnostic indicators of the action of testis hormone on the accessory organs. Studies of Golgi material were made from preparations by the Ludford modification of the Mann-Kopsch technique (1925, 1926), both the short and long methods being employed. Paraffin sections were utilized throughout. Other details of technique will be indicated in appropriate places.

[1] This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago. The author desires to express his appreciation to Dr. Carl R. Moore for his generous help and guidance during the course of the investigation.

GENERAL GROWTH OF REPRODUCTIVE PARTS

The growth tendencies of various parts of the male reproductive tract were determined from tissues collected from 73 animals of known ages ranging from 9 days to

TABLE 1

TABLE OF BODY AND GLAND WEIGHTS

Age (Days)	No. of Cases	Body Weight (Grams)	Weight (Milligrams)				
			Testes	Seminal Vesicles	Vas deferens Epididymis	Prostate Lobes	
						Middle and Posterior	Anterior
9	4	115	100.2	43.4	95.0	35.6	7.0
10	2	149	134.2	105.3	128.4	57.5	23.0
11	1	118	105.4	39.6	85.6	33.0	11.4
12	2	120	79.6	46.0	79.9	34.0	7.7
18	5	128	118.8	44.6	99.3	40.6	10.1
20	1	214	195.4	208.2	159.4	103.0	28.6
21	1	230	274.6	268.4	146.0	112.4	50.0
22	2	146	135.0	60.0	110.2	46.1	13.2
25	1	190	117.4	76.4	124.0	50.0	14.2
27	1	196	197.4	89.6	155.8	74.4	34.0
29	2	208	250.3	226.0	162.5	91.3	35.8
30	2	192	232.1	94.9	96.1	38.7	18.0
31	3	195	236.0	108.1	119.9	46.0	15.3
35	2	266	366.4	425.1	192.4	106.6	59.0
38	1	256	313.4	314.6	198.6	125.8	62.0
42	1	232	205.8	191.6	155.6	103.4	33.4
45	5	245	430.8	226.6	175.2	86.7	40.5
47	2	350	624.0	598.1	241.2	167.9	77.8
49	3	280	509.4	853.3	218.0	184.7	107.6
50	2	300	698.0	984.8	240.1	183.7	111.6
55	1	276	478.4	568.4	190.0	116.4	65.8
56	1	321	758.6	691.8	254.8	197.4	104.6
58	2	279	626.4	692.6	301.5	189.3	72.6
59	1	284	544.0	890.0	273.4	214.2	120.0
61	1	292	634.0	769.8	237.2	195.0	67.0
71*	1	270	242.2	82.8	178.2	63.4	23.8
72	1	308	1128.2	378.4	314.6	166.2	112.0
74	2	362	1389.5	510.0	324.0	222.7	101.2
77	2	360	1418.0	596.1	332.3	178.0	105.9
79	1	240	452.0	143.4	187.2	92.2	31.0
82	1	348	1072.0	430.0	256.6	171.4	78.0
83	3	377	1304.8	557.1	321.2	150.3	139.3
84	4	366	1161.5	673.4	303.2	181.8	102.6
85	2	311	1007.9	415.6	272.4	160.3	87.2
87	1	382	1426.8	437.4	318.2	184.6	87.4
91	1	305	975.7	600.0	243.2	163.6	81.5
98	1	299	1231.1	718.9	305.6	116.5	57.0
99	1	310	905.4	192.3	249.7	93.2	38.6
102	1	294	665.4	267.6	206.3	88.6	51.5
123	1	494	2239.0	1132.8	618.8	276.2	168.4
130	1	464	2025.8	822.2	497.2	241.4	157.0

* All animals including and above this age were ejaculated previous to killing by at least 2 days.

130 days old; by the latter age the male has attained its reproductive function. These data are presented in Table 1 and Figure 1. Seminal vesicles from animals older than

70 days are omitted from the graph, for the reason that all animals of this age and older were subjected to an electrical stimulation to induce an ejaculation; hence, a discrepancy in growth-rate is introduced.

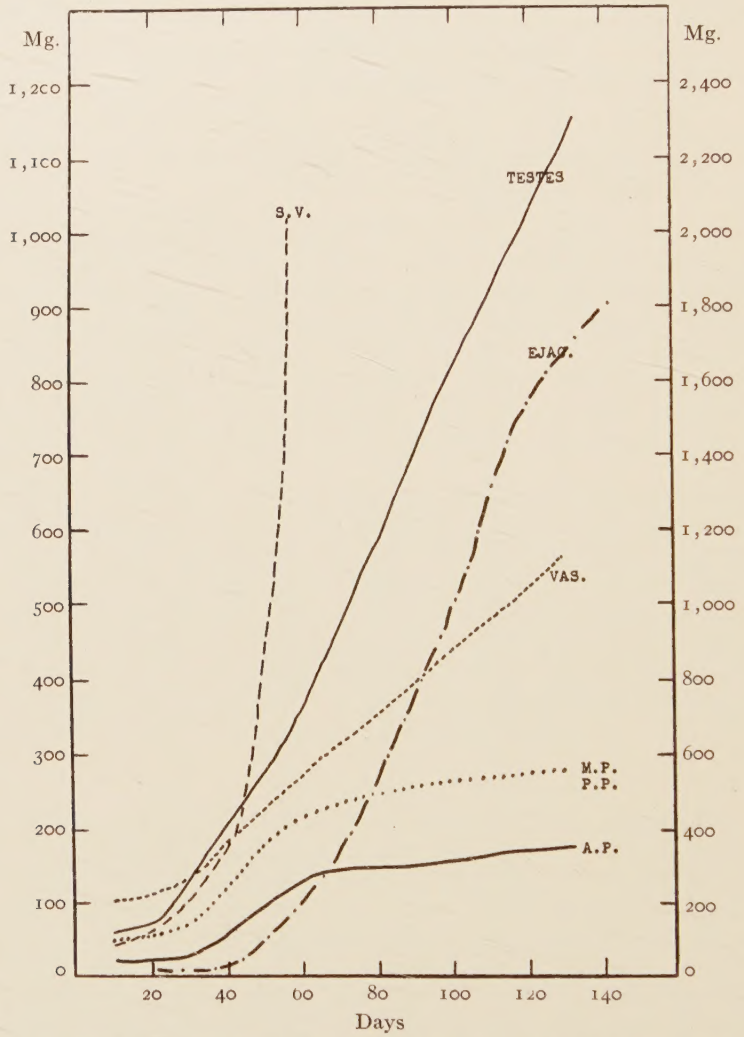

FIG. 1.—Growth curves of reproductive gland weights in normal animals and weights of ejaculates of prepuberal guinea pigs. Curves drawn by inspection from scatter of points of data in Table 1 and from scatter of points of the weights of 244 electrical ejaculates of normal young animals. Testes and ejaculate weights plotted after the right-hand scale; others, after the left-hand scale.

Aside from details of individual growth increments, perhaps the most striking point is the tendency of all organs to show a pronounced acceleration in weight beginning about the thirtieth to fortieth day of life. Testis increases occur slightly earlier than those of the accessory reproductive organs.

Since a detailed comparison of weights of individual accessory organs with general

body-growth shows a decidedly disproportionate growth on the part of the internal organs, one is immediately impressed with the indications of some critical period in the life of the organism at about this period. Testis weight, though increasing at a more rapid rate than general body-growth, exhibits a smoother general growth than the accessory organs; hence, the initiation of, or enhancement of, testis hormone secretion is probably responsible for the rather sudden accelerated growth of these accessory parts.

FUNCTIONAL DEVELOPMENT OF PROSTATE AND SEMINAL VESICLES

Seminal vesicles of most rodents produce a secretion that sets in a firm coagulum when mixed with prostatic secretions, and in the guinea pig the ejaculate forms a coagulated vaginal plug firmly attached to the walls of the vagina. The secretory function of these accessory organs has been shown to be dependent upon the internal secretion of the testes (Camus and Gley, 1896; De Bonis, 1909; Walker, 1910; Moore and Gallagher, 1930).

It was demonstrated by Battelli (1922) that male guinea pigs experience an ejaculation when stimulated on the head by an alternating electric current, and Moore and Gallagher (1930) have employed the technique as a means of studying the influence of testis hormone in the animal. It appeared advisable in this study to utilize the reaction as a means of determining the onset of function of the prostate and seminal vesicles in young animals, which would, in turn, establish at least the period in testis hormone secretion at which the effective threshold of hormone concentration was reached to induce this function.

Fifty-five guinea pigs were stimulated with electric shocks between the ages of 11 and 89 days after birth; and in case the initial stimulation failed to evoke an ejaculation, subsequent stimulations were given at weekly intervals until the first ejaculation was obtained. These stimulations followed the method of Moore and Gallagher and consisted on each occasion of a series of three shocks of 10 seconds each with approximately a minute interval between shocks. For smaller animals an alternating current of 6 volts was sufficiently strong, and graduated doses were utilized until animals of a weight of 350–400 gm. received between 25 and 30 volts. One needle-point electrode was inserted at the base of the skull just dorsal to the foramen magnum, whereas the other blunt terminal was inserted into the mouth. The collected ejaculate was weighed and examined for coagulation and for spermatozoa.

An analysis of the data shows that coagulable ejaculates may be obtained from animals as early as day 21; at this age a male weighing 194 gm. produced a coagulable ejaculate of 10 mg. The majority of males void a coagulable ejaculate by the thirtieth day of life, since 9 of 11 males receiving their first stimulation on day 26 produced coagulable ejaculates; these ejaculates varied from 1 mg. in weight to 150 mg., or an average of 68 mg. One male failed to void an ejaculate until the seventy-fourth day of life, and all stimulations at ages later than this yielded typical coagulable ejaculates. Analysis of the entire data on this point reveals that, among animals receiving stimulations between ages of 21 and 27 days, 45 per cent produced ejaculates; ages 28–34 days, 61 per cent; ages 35–41 days, 71 per cent; ages 42–48 days, 85 per cent; ages 49–55 days, 93 per cent; over 75 days, 100 per cent.

The curve of weight of the ejaculates shown in Figure 1 exhibits a gradual acceleration up to the oldest animal, tested on day 142. The amount of discharge at this time is essentially within the range of normal for adult animals, since Moore and Gallagher

reported 1.93 gm. discharge as the average weight of the second ejaculate; and in the case of the oldest animal here recorded, the weight was 1.75 gm.

Effects of prepuberal castration.—In order to study further the effects of testis hormone on the function of the prostate and seminal vesicles, as evidenced by the electrical ejaculation technique, a series of 15 young males were castrated and subjected to electrical stimulation. With the exception of 2 males, castrated at 5 and 8 days of age, all animals had been subjected to electrical stimulation at least once prior to castration.

Omitting details on individuals, the following results were obtained from this series of animals. (1) All males that had failed to give a coagulable discharge upon electrical stimulation pior to castration failed entirely to produce an ejaculate subsequent to castration; this was true despite stimulations given during several weeks after operation. (2) Animals castrated prior to day 40 failed entirely to deliver an ejaculate subsequent to operation. (3) Animals producing ejaculates prior to operation never again produced an ejaculate equal in amount to that delivered on the day on which castration was performed. (4) All animals failed to produce more than two coagulable ejaculates subsequent to castration, stimulations being administered each seventh day after operation.

Thus the average normal male produces a coagulable ejaculate by 36 days after birth, in an amount of 45 mg.; and the amount increases each week until maturity is established. The castrated animal either fails to produce ejaculates, in the event this function had not already been established before operation, or exhibits immediate marked regression in this function.

APPEARANCE OF SPERMATOZOA IN THE EJACULATE

The appearance of germ cells in the developing animal may be indicated from histological examination of testes, from smear preparations of an excised epididymis, or from age at first fertile matings. Since an ejaculation induced by the electrical method involves a reaction of all parts of the tract similar to that occurring under normal mating conditions, it was established as a routine to examine all discharges in saline solution in order to determine the age at which spermatozoa are discharged sufficiently mature to exhibit a normal type of motility. It is again to be emphasized that stimulations were given each seventh day after the initial stimulation. No attempt has been made to determine the age at which fertility is established.

Among the 55 young males whose ejaculation history was closely followed, a considerable degree of variability is found in the first discharge of spermatozoa. Fifty-four days was the earliest and 116 days the latest age at which first discharge of spermatozoa occurred; the latter animal had been subjected to fourteen consecutive weekly stimulations before spermatozoa were obtained in the discharge. More than one-half of the males voided spermatozoa by an age of 75 days, and only one reached an age greater than 100 days before spermatozoa capable of motility were obtained.

The data obtained from living healthy animals, and supplemented by a study of fixed materials (in subsequent sections), thus indicate the progression in acquiring normal reproductive functions. The average animal is capable of voiding its first coagulable seminal discharge on day 36. It becomes evident, therefore, that seminal vesicle and prostate function are acquired considerably earlier than the formation of spermatozoa, which occurred on day 54 at the earliest and on day 75 in one-half the males examined. Should one define "puberty" (as distinct from "maturity") as the time at which sperma-

tozoa capable of motility can be found in a typical coagulable seminal discharge, one could say that puberty is attained in 50 per cent of male guinea pigs by day 75 and in practically all males by an age of 115 days. The average animal at puberty will void a coagulable ejaculate of 300–400 mg. and will possess a body weight of 335 gm.

HISTOLOGICAL STUDY OF THE ORGANS OF THE REPRODUCTIVE SYSTEM

The foregoing account of the general growth-trends of various parts of the reproductive system and the study of the time of attainment of the various functions in the living animal have been closely correlated with a study of the histology of the various portions concerned. In this manner one is on somewhat more secure grounds in offering a physiological interpretation of structural conditions found in fixed preparations. The study of a close series of gonads and accessory organs should therefore be of some interest, especially since the tissues have been correlated with known physiological states determined on the living animal.

Testis.—Certain major features of interest in testis differentiation emerge from a close study of the organ from 77 males sacrificed at an age-range of from 2 to 130 days.

Prior to day 20 the testis consists of solid cords containing inactive spermatogonia and indifferent cells. A seminiferous tubule lumen appears in the majority of testes, from day 30 to day 40, and spermatocytes are present. Spermatids appear from day 45 to day 60, and metamorphosing spermatids ("sperm heads" of Moore, 1936) or spermatozoa were present in 1 case out of 13 between days 40–50, in 2 cases out of 10 between days 50–60, in 4 cases out of 5 between days 60–70; spermatozoa were present in the tubules almost invariably subsequent to day 70.

Spermatozoa are to be found in the epididymis shortly after sperm heads or spermatozoa appear in the testis. Although the earliest epididymal smear showing spermatozoa occurred between days 60 and 70, it will be recalled that in one case of electrical stimulation spermatozoa were present in the ejaculate on day 54: this specific animal was not sacrificed until day 62. Spermatozoa were usually present in the epididymis after day 70.

These observations, while showing a fairly rapid progression in the differentiation of germ cells in the testis, reveal that a greater discrepancy occurs in guinea pigs than in rats as to consistency in terms of age of spermatozoan differentiation (see Moore, 1936).

Seminal vesicles.—The seminal vesicles of 23 normal animals sacrificed at periods from 2 to 80 days of age were carefully studied histologically to determine in general the sequence of conditions from nonfunctional to the fully functional organ. This was supplemented by a study of the same organ from 67 animals castrated during this developmental period and sacrificed at varying periods thereafter. Thus, for example, 12 males were castrated between the fifth and eighth day of life and sacrificed periodically from day 15 to day 130. The total range of cases involved castrations at ages 1–73 days with sacrifices thereafter up to an age of $2\frac{1}{2}$ years.

In general appearance the cells of seminal vesicles of animals of 2–6 days of age are tall and columnar, tending in some limited areas to show a pseudo-stratified condition. The nuclei, in general, occupy a basal position; but the general cytoplasm fails to take a counterstain, excepting that the plasmalemma does stand out sharply. A transitional picture appears in animals 10–30 days of age. Many epithelial cells of the seminal vesicle have acquired a state that permits the cytoplasm to stain quite as does that of mature normal vesicles. Subsequent to day 30, the general appearance of the epithelium does not differ materially from the adult condition.

Castration at day 5 does inhibit the development of this general staining reaction of the epithelial cells, since sacrifice at day 45 or later reveals that the cells stain in accordance with the normal pattern of the 5-day-old pig.

Careful measurements of the epithelial height of seminal vesicles of all categories have been made by means of an ocular micrometer, to determine, first, the progression during normal development and, second, whether castration offers a modifying factor in development.

The results of the study of cell height have been somewhat surprising in the lack of any definite trend in effect, either in normal development or from castration. Variations in cell height determined from 23 normal males at ages of 2–80 days presented a range from 17 μ to 25 μ, or an average of 20 μ. Individual ranges have given no consistent increment in height, since in vesicles from animals 2 days of age cell height was greater than in vesicles removed from animals 60 days of age. The average cell height has therefore been attained by 2 days after birth.

Changes in cell height as a result of castration have been surprisingly inevident and inconsistent. One indication of involution in seminal vesicle epithelium of castrates occurs in cases where castration of short duration only begins at approximately the age of establishment of secretory function. Thus, an animal castrated at day 35 and sacrificed 10–20 days later may show an epithelial height of 11–13 μ, which is lower than any normal male in the series. On the other hand, another animal castrated on the same day but permitted to live for more than 2 years may show an epithelium appreciably higher. Thus, temporary reduction in cell height is suggested, but such reduction fails to characterize clearly a castrate condition.

Golgi apparatus.—The lack of any specific cell inclusion capable of definite diagnosis of the secretory state of the seminal vesicle of guinea pigs, such as portrayed by the large "secretion granules" of rat seminal vesicles by Moore, Hughes, and Gallagher (1930), suggested a study of the general responses of the Golgi body. The above-mentioned authors found definite castration modification in the Golgi bodies of the rat tissues.

The study of the progressive development of the Golgi body in seminal-vesicle epithelium of normal guinea pigs reveals that up to approximately 20 days of age the tall columnar cells contain a few osmicated granules either scattered or clumped in the lumen end of the cells. In slightly older males the cells reveal a more extensive granular state. The granules may be grouped in the lumen end of the cell, or occasionally the cytoplasm peripheral to the nucleus may be quite filled with osmophilic granules. By the forty-fifth day of life the previous copious supply of granules becomes aggregated into a knot approximately 8 μ in height and composed of a mass of coiled threads, usually running approximately parallel to the long axis of the cell. This condition characterized the cells of postpuberal and mature males.

Castration induces some modification in the differentiation of the Golgi body. In general it may be stated that the stage characteristic of normal males approaching maturity is not attained in animals castrated at an early age. Thus, castration prior to day 8 and sacrifice up to the forty-fifth day of age leave the Golgi apparatus in a juvenile state. The lumen end of the cell contains scattered osmicated granules or aggregates of granules. Animals castrated subsequent to the tenth day of age and permitted to live a year or longer still show reduced Golgi material in the seminal vesicle cells, but it is more typical of postpuberal castrates and may assume the form of a condensed nuclear cap. It becomes apparent that the histological condition of the seminal vesicles of the

guinea pig does not provide an adequate index of its functional state. Although electrical ejaculation proves that the vesicles have a normal function at an early age, there is a lack of clear histological condition to indicate it. There is an absence of cellular inclusions (well-defined granules) or of cell-height increase (or decrease after castration) that provides a reliable criterion for presence or absence of testis hormone.

Anterior prostate gland.—Studies on this portion of the prostate gland have been directed toward progressive differentiation with onset of its normal function and to effects that may be evident from castration; it is to be realized, therefore, that its structure in relation to function has been foremost in the course of these studies.

When cell height of the epithelial lining of anterior prostate acini is considered, one soon realizes that little difference exists between the cells of the inactive gland and those of one fully active. Careful measurements of cell height in glands removed at an age of 2–70 days indicated a range of 16–25 μ, or an average of 21 μ; greatest cell height occurred in the case of an animal 10 days old, and the lowest in a 15-day-old male.

Certain epithelial changes indicate progressions in development. The acinus lumen of the 2-day anterior prostate was 0.074 mm. in diameter, and this was progressively larger until in a 73-day-old prostate the lumen was 0.59 mm. The youngest anterior prostate epithelium is a simple columnar type. This gradually changes, with approaching maturity, into a pseudo-stratified condition, which changes further into a highly folded type. No characteristic cell inclusion of diagnostic value in determining the onset of active secretion was seen.

Castration at early stages exhibited itself principally by rather slight changes in the character of the epithelium and by an increase in size of the acini. Cell height appears to be unmodified by loss of testes. The epithelium tends to be decidedly less folded, and the cells are usually crowded together, producing a pseudo-stratified or stratified condition.

The osmic-acid technique for Golgi-body studies failed entirely in my hands. In anterior prostates of both young and mature males methods that proved successful with other tissues were unsatisfactory. On the whole, therefore, changes that denoted functional activity on the part of this gland failed to appear in either the progressive development or as a result of castration.

Middle prostate gland.—The middle lobe of the prostate gland does not differ markedly in its reactions from the anterior lobe. There is a progressive development from the columnar epithelial lining of simple acini to the folded epithelium of larger acini. Cell height cannot be closely correlated with age, at least from birth to puberty; and variations from 16 μ to 28 μ yield a general average of 22 μ.

Castration likewise does not markedly affect the histological character of this gland. There is some suggestion of a diminution in epithelial height when the testes are removed about the seventy-fifth day, but so many discrepancies enter that one is incapable of definitely diagnosing castration on the basis of general histological changes. Thus, the middle prostate from an animal castrated on day 35 and sacrificed more than 2 years later consisted of epithelial cells that fell within the range of cell heights in normal males.

Osmic acid impregnation of this portion of the prostate was fully successful despite the somewhat disconcerting results in attempting to relate specific morphological responses with a particular functional state. A definite Golgi body is present in the apical portion of middle lobe cells, and its general morphology in animals a few days old is

quite comparable to that of mature males. The body usually consists of a mass of coiled threads oriented in the long axis of the cell between nucleus and acinus end of the cell. Impregnation appears to be less intense in animals previous to 30 days of age than in mature males.

Golgi-body changes resulting from castration are more definite than is cell-height modifications. Castration between days 23 and 60, with sacrifice 100 days later, induces consistently a reduced Golgi body in the form of a cap against the nucleus of the cell. When operations were done from 2 to 8 days after birth, osmophilic material was present as delicate broken or continuous threads or as scattered granules. This suggests an inhibition of normal development in the Golgi body. The principal effects from castration, therefore, are reduction in size and in intensity of impregnation with osmic acid.

Posterior prostate.—A study of the posterior lobe of the prostate similar to that reported in slightly more detail for the anterior and middle lobes yields results approximating those obtained from a study of those portions of the gland. The lumina of the posterior-lobe acini are fairly characteristic in showing a progression from a diameter of 0.074 mm. in the case of a 2-day-old animal to 0.37 mm. diameter in a 60-day male. Cell height is again too variable to offer a diagnostic criterion of function.

Castration appears to induce a temporary reduction in cell height, but in cases of the longer periods of castration the cells often fall within the range of the normal males. The Golgi-body reactions of long-time castrates are again variable, but there is a decided tendency toward a diminished intensity in impregnation. In general the Golgi-body impregnation is sharpest in this lobe.

Vas deferens.—The vas deferens was studied in progressive sexual differentiation and in castration, but it likewise fails to offer outstanding structural conditions that can be definitely associated with a functional condition. The lumen of the tube increases progressively from 0.18 mm. prior to day 15 to a diameter of 0.40 mm. in 60-day males. The epithelial folds increase gradually in height; the cytoplasm of the cells stains deeply; and the apical ends of the cells are quite irregular.

Castration tends to reduce the diameter of the lumen, but considerable variability exists. No appreciable change in cell height follows removal of the testicle, and diagnosis of secretory states in this structure are difficult from general histological appearances.

Golgi-body reactions do not clearly indicate a secretory state in cells of the vas deferens as sexual differentiation progresses. The body lies in the long axis of the cell, apical to the nucleus. It is entirely similar in cells from the 6-day male and normal adult male. Castrations, particularly when performed at about 45 days of age, induce fairly distinct and consistent reductions in the osmicated materials, since the normal large knotlike mass becomes, in the castrated animal, sparse osmophilic granules scattered in the cytoplasm peripheral to the nucleus, or it may take the form of a thick threadlike structure. It is clearly reduced despite the lack of cell-height reduction.

DISCUSSION

The observations here reported were derived from a study of the progression in postnatal sexual differentiation in the male guinea pig. This study involved: (1) the individual growth of various parts, as exhibited by determinations of fresh weights on

carefully dissected organs; (2) the histological condition from birth to a well-developed functional state; (3) the study of the organs after castration performed at various ages; (4) the utilization of such functional tests as the capacity of spermatozoa to exhibit motility, the ability of seminal vesicles and prostate gland to provide normal secretions making up a coagulated ejaculate, and the ability of the animal to void such an ejaculate when properly stimulated.

Considering first the general growth of the reproductive parts, one is impressed with the sudden growth stimulus which occurs slightly earlier in the testis (at approximately the twentieth day) than in the accessory organs of reproduction (about 30–40 days of age). The curves of growth representing these trends fail, naturally, to portray individual variability; hence, analyses of individuals in respect to various aspects become enlightening. This analysis has been utilized both with respect to germ cell development and with respect to the internal secretory activity of the testes.

One of the most definite indexes of testis hormone secretion in the guinea pig is the voiding of a coagulable ejaculate, since this assures a functional state in both prostate and seminal vesicle, which function is under testis hormone control (see Moore and Gallagher, 1930). The observations reveal a fairly widespread variability in the attainment of this function in individual males. The youngest male to perform an ejaculation following electrical stimulation did so on day 21, whereas the oldest tested animal to first attain this capacity was 74 days of age. Fully one-half of the males delivered small coagulable ejaculates by day 30. This coincides approximately with the period of a rise in the curve of weights of the accessory organs. Thereafter both the growth curve and the curve of weights of ejaculate mounted upward until the early stage of maturity was reached. These responses therefore provide indications of the effectiveness of testis hormone.

The study of the progression in testis differentiation, carried on principally by histological study of the testes, was supplemented both by examining smear preparations of the epididymis and by an examination of the electrically induced seminal discharge for spermatozoa. Some variability again exists; but, on the whole, there is a fair consistency in the rate of germ-cell differentiation for all males studied. Metamorphosing spermatids (sperm heads) were first noted at an age of 48 days. This stage of development was attained by only 1 male among 13 examined between days 40 and 50; 3 among 10 males reached the sperm head or spermatozoan stage of development between days 50 and 60, while the majority of animals examined from 60 to 70 days had attained the same stage. One male only was found in which testes did not show spermatozoa at 70 days or later, and the age of this male was somewhat in question. On the whole, spermatozoa were not present in the epididymis until day 65, though one male voided a few motile spermatozoa in an ejaculation on day 54; motile cells appear in the ejaculate at approximately the same time as found in epididymal smears. In one case, only a few spermatozoa were found in the epididymis on day 73, when the ejaculate induced immediately prior to sacrifice failed to show them.

These considerations indicate a fairly rapid metamorphosis of germ cells, passage into the epididymis, acquisition of capacity for motility, and discharge through an ejaculation. Seventy days after birth represents a stage of development in the male guinea pig when the great majority of animals will void coagulable ejaculates containing spermatozoa that are actively motile.

The microscopical study of the accessory organs of reproduction yielded somewhat

surprising results in that structural conditions of the various parts change very little with the onset of function in normal progressive differentiation or with the loss of hormone following removal of the testes. A greater rapidity and extent of structural change with modifications in the hormonal state would be expected to be more apparent in this rodent in the light of such outstanding changes in the rat and ground squirrel (see Moore, Price, and Gallagher, 1930; Moore, Hughes, and Gallagher, 1930; Price, 1936; Wells, 1935). Whereas in normal differentiation the general character of the cytoplasm gives some indication of the changing functional conditions, cell height is modified but little. The height of the mature epithelium in many of these organs has already been attained at 2 days of age, and in early or later stages of castration there is only slight modification. Histological detail does not readily permit distinguishing the accessory reproductive organs of a male castrated longer than a year from those of normal males. In no technique employed by me have distinctive or diagnostic cell inclusions been found that were capable of denoting a definite functional state on the part of these accessory glands of reproduction. The condition of the Golgi bodies has most nearly approached this capacity. In certain portions (posterior prostate, and vas deferens) a clearly progressive differentiation was evident; the conditions are more or less characteristic of each tissue studied, and in some of these castration induced fairly evident changes from the normal. In other organs, however, progressive development failed to present apparent differences, and castration often produced such slight changes as to make it almost impossible to diagnose the state as one of lack of function lost through castration. The guinea pig, therefore, is a poor test animal for the study of effects of testis hormone on the histological state of the accessory reproductive organs. The electrical ejaculation test, on the other hand, and possibly also the spermatozoan motility test (not used by me; see Moore and McGee, 1928), is a useful test method.

In a later paper more detailed studies will be reported from the study of the effects of castration in mature animals.

SUMMARY AND CONCLUSIONS

1. Growth-rates of testes and accessory reproductive organs of male guinea pigs are accelerated from days 30–40; thereafter growth is rapid.

2. Ejaculations are induced as early as day 21, and the majority of males demonstrate this capacity by approximately day 30.

3. Spermatozoa first appear in the testis about day 50; they are fairly uniformly present by day 70. Spermatozoa appear in the epididymis and are discharged in an electrically stimulated ejaculate within a few days from their first appearance in the testes.

4. Histological study of accessory organs of reproduction during the age of functional development shows a lack of progression in cell-height increases. Prepuberal castration exerts but little modification in prostate and seminal vesicles. Some tendency for lowered cell height is suggested in castrations subsequent to about day 30. No specific cytoplasmic inclusions of diagnostic value for the process of secretion have been noted. Golgi-body changes provide some evidence of lack of testis hormone but are too variable to be of diagnostic value.

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THE EFFECTS OF CASTRATION UPON THE REPRODUCTIVE ORGANS OF THE ADULT MALE GUINEA PIG [1]

E. DUANE SAYLES
The University of Chicago, Illinois, and Virginia Junior College, Minnesota

TWO FIGURES

The experimental work of many investigators has shown that castration of the male rodent results in the involution of the accessory reproductive organs. This has been shown to be the case particularly in the rat, mouse, thirteen-lined ground squirrel, and in part in the guinea pig. With the involution of these glands cytological and histological changes have frequently been rapid and diagnostic following loss of the normal hormone supply. The accessory organs studied in this report are the long, tapering seminal vesicles, the compound tubular anterior, middle and posterior lobes of the prostate and the ductus deferens. Cowper's gland was not studied because Heller ('32) found that it underwent retrogression following castration.

An earlier paper (Sayles, '39) reported that in the young normal guinea pig the accessory reproductive organs gradually increase in weight up to 30 days of age following which there is a marked acceleration in rate of the weight increase. Spermatozoa do not appear in the testes until 77 days of age. The accessory organs show adult histological characteristics at the age of 30 days and tend to give the characteristic adult castrate response in animals castrated after this age.

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With these facts in mind an attempt has been made: (1) to study the gland weights of normal and castrated adult guinea pigs; (2) to study the normal histology and cytology of the accessory glands of the adult and, (3) to study the changes that might appear in these glands as a result of castration.

MATERIALS AND METHODS

One hundred and two vigorous adult male guinea pigs have been used. Most animals were kept isolated from females. Castration was performed through an abdominal incision. Small pieces of tissue were taken at autopsy and immediately fixed. For general study, the fixative consisted of normal saline solution saturated with mercuric chloride, to each 100 cc. of which 5 cc. of glacial acetic were added. For demonstration of Golgi bodies the tissues were immersed in Mann's fluid and impregnation was completed with Ludford's modification of the Kopsch technique ('25, '26). All tissues were cut at 4 μ. Ehrlich's haematoxylin and eosin were used as general stains. All prepared slides were studied with an oil immersion lens and measurements were made with an ocular micrometer. In determining cell height and Golgi body size an average was obtained from fifteen measurements of typical epithelial areas. This value is the one used in the tables and in the body of the paper. In determining the lumen diameters, the sections for each animal were examined under low power and the tubule lumina measured until the maximum measurement was considered to have been obtained. Other details of procedure will be noted in the appropriate places.

PROPORTIONATE WEIGHTS OF REPRODUCTIVE ORGANS OF NORMAL AND CASTRATED ADULTS

Thirty-five adult guinea pigs of known weights and approximate ages were used in this study (table 1). Organs of these animals were not used for histological study. Eleven normal guinea pigs were sacrificed and their fresh glands dissected under the dissecting binoculars and immediately weighed on a chainomatic balance. The remaining animals were castrated and allowed to live for 15, 33, and 88 days respectively before

determining the weight changes due to castration. The weights of the normal sex glands tend to keep pace with general body growth, at least up to the 600-gm. weight animals.

TABLE 1

Table of body and gland weights. Adult guinea pigs.

DAY AFTER CASTRATION			NO. OF CASES	BODY WEIGHT (GM.)			GLAND WEIGHT (MG.)			GLAND WEIGHT PERCENTAGE OF BODY WEIGHT
Low	High	Average		Low	High	Average	Low	High	Average	
				Seminal vesicles						
Normal			11	441	710	531.8	1073.0	4365.0	2467.4	.466
15	15	15	6	478	754	555.6	385.0	1792.8	960.8	.17
33	36	33	9	406	596	511.8	351.5	732.7	605.8	.118
72	125	88	9	474	730	631	371.4	643.0	525.5	.098
				Anterior prostate						
Normal			11	441	710	531.8	136.1	574.0	246.3	.046
15	15	15	6	478	754	555.6	122.8	293.6	165.9	.029
33	36	33	9	406	596	511.8	75.3	136.4	106.1	.020
72	125	88	9	474	730	631	104.5	204.8	159.0	.025
				Middle prostate and posterior prostate						
Normal			11	441	710	531.8	108.2	592.4	315.8	.059
15	15	15	6	478	754	555.6	181.6	405.6	279.8	.050
33	36	33	9	406	596	511.8	112.6	270.1	191.6	.037
72	125	88	9	474	730	631	93.9	316.4	188.6	.029
				Entire prostate gland						
Normal			11	441	710	531.8	264.2	990.3	562.1	.105
15	15	15	6	478	754	555.6	304.4	699.2	445.8	.08
33	36	33	9	406	596	511.8	195.3	390.4	286.7	.056
72	125	88	9	474	730	631,	225.9	430.7	347.6	.055
				Vas deferens and epididymis						
Normal			11	441	710	531.8	565.0	1267.0	935.3	.175

The effect of castration upon gross changes in the glands is most strikingly shown in the seminal vesicles (fig. 1) in that after 15 days the average weight of the glands and contents is but about one-third that for normal animals. This great drop is largely due to reduction in the amount of secretion and not to real loss of tissue. Longer periods of castration show further reduction with regression nearly complete at 33 days after castration.

In an attempt to equalize the data in the light of the varying body weights of the animals used, the average gland weights have been expressed in terms of the per cent of average total body weights. For the seminal vesicle, table 1, the average

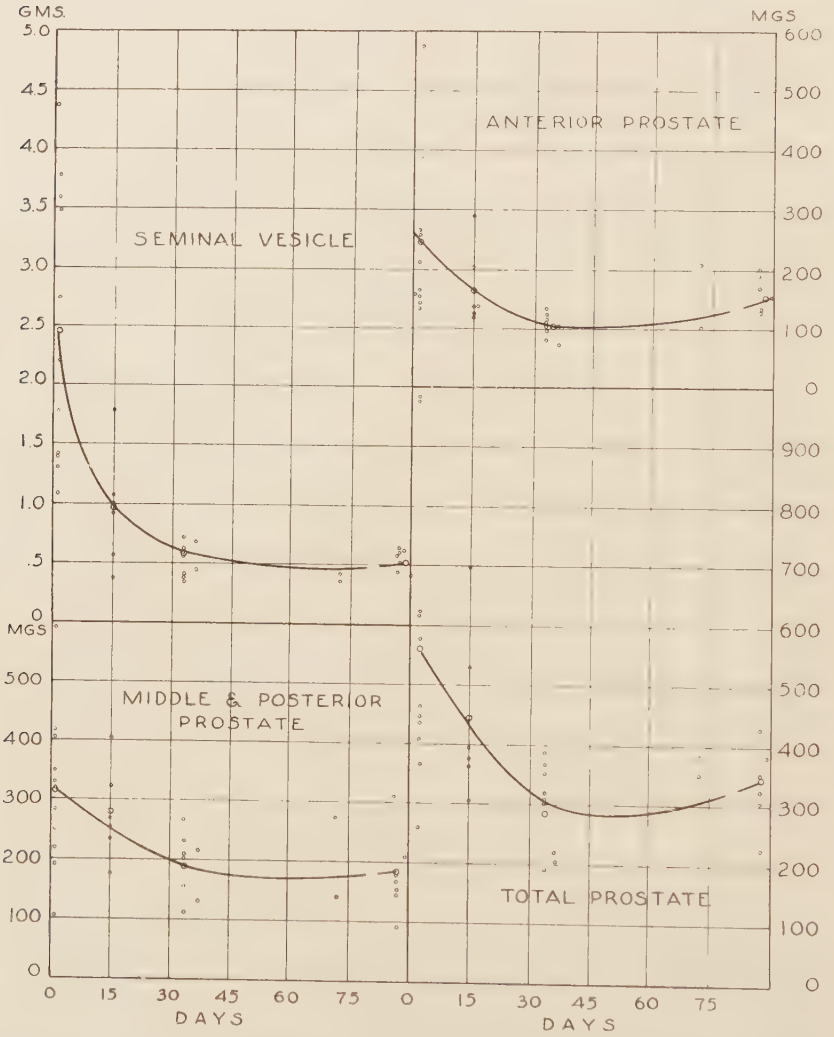

Fig. 1 Regression in weights of accessory glands following castration. From data summarized in table 1. All points shown after the break in the curve are for animals castrated for more than 75 days.

normal seminal vesicle with secretion is .466% of the weight of the average animal of 531 gm. By 15 days after castration this value is reduced to .17% of the average castrated guinea pig weighing 555 gm.; this is further reduced by 33 days to .118% of the average castrate 511-gm. animal and to .098% of the average 631-gm. guinea pig at 88 days after castration.

Castration produced a reduction in the weight of the prostate complex of 50% in animals castrated for 88 days. The anterior prostate appears to undergo regression more quickly than the rest of the gland for the data indicates that by 15 days this reduction is nearly complete while the middle and posterior portion is reduced but one-third of normal at 33 days with further reduction by 88 days of castration.

HISTOLOGICAL STUDY OF NORMAL AND CASTRATED ANIMALS

Seminal vesicles

Walker ('10) describes the seminal vesicles as follows. The glands are free except for their attachment at the urethral end. The tubules consist of longitudinal and circular muscle coats which are lined with columnar epithelium. The secretion is a clear tenaceous fluid. Warnock ('23) emphasizes the epithelium as forming folds which spiral from the base to the distal end. Armisted ('25) indicates the lining cells tend to be two and one-half times as high as they are wide. The average cell height in this report of 21 μ is about five times the cell width. The cell width is seldom greater than that of the nucleus. The latter is basophilic showing chromatin masses within the definite nuclear membrane. The eosinophilic cytoplasm stains quite uniformly with the lumen margin taking a slightly more intense stain than any other part of the cytoplasm. In contrast to the juvenile normal and castrate picture the cell membrane seldom takes a stain any more intense than that of the general cytoplasm. The epithelium is supported by a submucosa which extends the full depth of the folds.

The thickness of the muscle coats of the normal and the castrate glands was compared and no significant difference

was evident. Lumen diameter measurements were impossible because only small pieces of tubule were fixed.

Table 2 summarizes the cell heights for each of the glands of the animals studied. The epithelial cell height was found

TABLE 2

Epithelial cell heights of the accessory reproductive organs of the male guinea pig.

DAYS AFTER CASTRATION Low	High	Average	NUMBER OF CASES	HEIGHT IN MICRONS High	Low	Average
			Seminal vesicles			
Normal			16	25	18	21
5	15	10	14	20	13	17
20	26	22	14	18	13	15
30	32	30	6	17	12	14
41	50	47	6	16	10	13
75	605	392	9	13	10	11
			Anterior prostate			
Normal			14	23	17	21
6	15	11	13	25	16	21
20	26	20	14	27	17	21
30	32	30	5	21	18	20
41	50	47	6	27	17	21
75	605	378	8	23	13	19
			Middle prostate			
Normal			16	26	16	21
6	15	11	13	23	12	17
20	26	20	12	22	13	16
30	32	30	6	16	10	13
41	50	47	6	17	11	15
75	605	392	9	14	9	11
			Posterior prostate			
Normal			15	23	17	20
6	15	11	13	22	12	17
20	26	20	13	21	13	17
30	32	30	6	21	13	16
41	50	47	6	21	12	17
75	605	366	8	22	12	14
			Vas deferens			
Normal			16	29	15	24
6	15	10	10	26	18	21
20	26	21	10	24	17	19
30	32	30	5	20	15	18
41	50	47	6	27	16	19
75	605	346	7	23	13	17

to be relatively constant in the normal animal varying from 18–25 μ, averaging 21 μ.

Castration effect upon cell height in the seminal vesicle epithelium is noticeable in most animals within 5 days after castration (fig. 2). Those animals castrated for 30 days

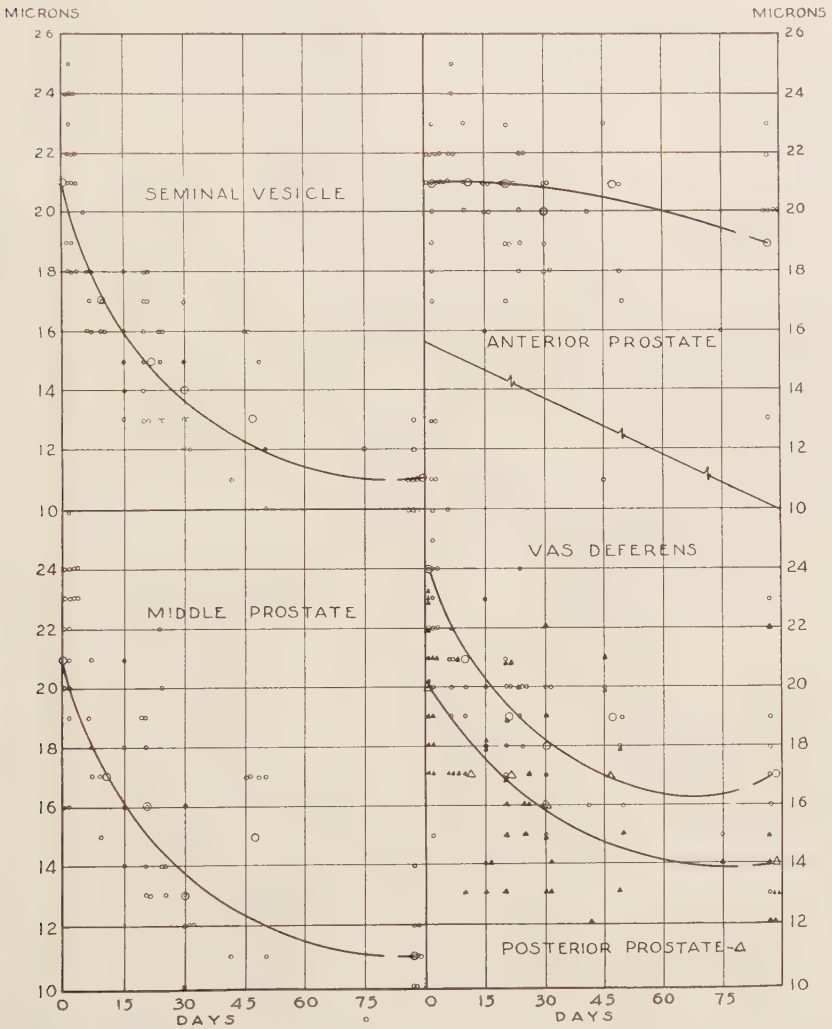

Fig. 2 Regression curves of epithelial cell heights drawn by inspection from the scatter of points of the data of sixty-seven animals. Ordinates in microns; abscissae in days post operative. All points shown after the break in the curve are for animals castrated for more than 75 days. From data summarized in table 2.

approach the condition of the long time castrates. That this regression in cell height is not uniformly completed by 30 days is shown in the records of the guinea pigs castrated for 45–49 days which are higher than those of the 30-day period. The average cell height of the nine animals castrated for periods from 75 to 605 days (hereafter referred to as long time castrates) is 11 μ. The range of the variation is between 10–13 μ. Castration of the adult guinea pig results in a reduction of the epithelial cell height in the seminal vesicles of approximately one-half.

The Golgi apparatus definitely changes in form following castration. This impregnated body in the normal seminal vesicle epithelial cell has the form of an osmicated thread knot 4 to 10 μ in height. Its position is in the middle third of the cell well separated from the nucleus. Castration changes appear within 5 days, at which time large knots are no longer evident. The typical body now measures but 4–5 μ. In many of the short time castrates the osmicated parts are granules scattered in the cytoplasm of the cell. This may be the result of some unnoticed variation in the technique. Reduction in the size of the Golgi body is progressive until in the 30-day post operative animal it has reached the minimal form, a low mass of osmicated threads which is applied to the lumen end of the nucleus in the form of a nuclear cap. Golgi body reduction following castration then is from a knot of 8 μ in height to a nuclear cap which seldom exceeds 1 or 2 μ.

Anterior prostate

The anterior prostate is lined with a columnar epithelium in the form of villi and folds extending deeply into the large lumen of the adult gland. The epithelial folds may anastomose in many places and are supported throughout by a heavy circular muscle layer and by longitudinal muscle fibers. The nuclei of the epithelium are nearly round, measuring slightly less than 8 μ in their greatest diameter. Chromatin granules are not numerous. The cytoplasm takes a delicate uniform

eosin stain. The epithelium is commonly bordered by large eosinophilic globules while the remainder of the lumen is filled with secretion matter.

The acinus of the normal anterior prostate has, on the average, a maximum lumen diameter of .5 to .6 mm. For nine animals castrated for periods from 75 days up to 19 months the maximum lumen diameter was .34 mm., indicating a reduction of one-third in lumen diameter in the long time castrates. There is no significant reduction during castration periods of less than 50 days.

As can be seen in the data and in figure 2, the epithelial cell height fails to be significantly modified by castration. A histological alteration of variable nature is evident in some castrate tissues. The number of cells in the epithelium apparently becomes less for numerous cells are cast into the lumen with the remaining cells greatly distended, many assuming a globular form. In the folds of the epithelium the cells do not stand out at right angles to the supporting layer but assume an oblique angle with it.

Despite considerable effort to find a suitable technique for the Golgi apparatus of the anterior prostate no satisfactory results were ever obtained.

Middle prostate

A section of the middle prostate shows a tubular construction similar to that of the anterior prostate. The muscle coat is not as thick as in the latter. In staining reaction the epithelial cytoplasm is more basophilic and the nuclei are more oval than in the anterior gland. The lumen diameters of the middle prostate show some castration effects. Measurements of maximum lumen diameters of the normal glands average .297 mm. while those of the long time castrates average .199 mm. The reduction then is about one-third. In most cases the reduced lumen diameter is approached after 40 days of castration with an average maximum lumen diameter of .216 mm.

Epithelial cell height changes are not uniform but do show definite castration effects. Cell heights of the sixteen normal animals varied from 16–26 μ with an average of 21 μ. Castration effects upon the cell height are evident in the majority of cases within 7 to 10 days after castration. The minimal condition is not approached until 30 days following castration. Even at this late period the results are confusing due to the non-conforming cases of the animals castrated for 45–49 days. Although some of these measurements run higher than those of the 30-day castrates it is to be noted that they are well below the cell heights of the adult normal non-distended tubules. Middle prostate epithelial cells of the nine long time castrated animals show cell heights varying from 9–14 μ, averaging 11 μ. The one-half reduction in cell height is usually attained by 30 days following castration.

The Golgi body in the cell of the secretory epithelium is similar to that of the seminal vesicle. It varies in size from 4 to 8 μ. Castration is not followed by uniform changes in the Golgi bodies of the middle prostate. Some short time castrates show 8 μ knots; others of equally short duration show the nuclear cap picture. Tissues of some animals castrated for 30 days show the normal type knot; however, the long time castrates show a constant picture of the low nuclear cap type of Golgi body.

Posterior prostate

The histology of the posterior prostate is similar to that of the middle prostate. Lumen diameters are less and the muscle coats are thinner than in the previous lobes. The intensity of the eosinophilic staining of the epithelial cytoplasm is approximately half way between that of the other two lobes.

Castration results in a one-third reduction in the lumen diameter. The average normal maximal lumen diameter of .319 mm. becomes reduced to .237 mm. in animals that have been castrated for 30 days. There is little reduction after the 30-day period.

Castration changes are marked in the epithelial reduction of the posterior prostate. Cells in the normal animals vary in height from 17 μ in distended tubules to 23 μ. The average for the fifteen animals studied as normals was 20 μ. Cell height loss is evident in many of the animals by 10 days after castration. The majority of the castrated guinea pigs show a regression in cell height which approaches the maximum by 30 days; the average of seven long time castrates being 14 μ.

Golgi body pictures are most striking and most uniformly impregnated in the posterior prostate lobes. Normal tissues show a large knot, as large as 12 μ, free from the nucleus in the middle third of the cell. The most common size of the Golgi body is 8 μ. Castration effects are evident 5–10 days after castration for usually the bodies drop to a 4 μ knot-cap applied to the lumen end of the nucleus. Animals castrated for 20 days or longer quite uniformly show a knot-cap which rarely extends 2 μ from the nuclear membrane.

Vas deferens

The histological picture of the normal vas deferens is that of a heavily muscled tube, lined with a deeply folded non-ciliated columnar epithelium. The epithelium and the muscle coat are separated by an extensive submucosa layer the cells of which are frequently cuboidal in nature. This cuboidal layer may become especially pronounced in the castrated animal.

The maximum diameter of the cross section of the normal vas deferens varies between 1.50 mm. and 1.93 mm. The latter number was obtained from the study of a serially sectioned tubule while the former was the average of the other normal ducts. Castration changes were not diagnostic until after 50 days when the average was 1.18 mm.

The normal lumen diameter measurements varied from .4 to 1.03 mm. The average diameter determined was approximately 0.4 mm. The average lumen of the long time castrates is about 0.1 mm. less than that of the normal average. No significant difference appeared to occur in animals castrated for short periods of time.

Cell height changes have been one of the most striking effects of castration found in the vas deferens. In this normal columnar epithelium the cells range from 15–29 μ, averaging 24 μ. Within 10 days after castration the cell heights usually are less than 20 μ. The cells of the 30-day castrates are as low as those of the long time castrates. Among the long time castrates the cell height varies from 13–23 μ averaging 17 μ. Cell height reduction following castration is gradual and reaches the minimum by 30 days. A marked lowering of the epithelial folds plus the development of the definite supporting cuboidal layer in most castrates makes for very positive indicators of testicular hormone loss in the animal.

Golgi body studies in the vas deferens have shown pronounced changes after castration. In the normal tissue the impregnation is very intense with the rest of the cell appearing quite clear. Within a complete cross section of the duct the Golgi bodies form a continuous band in the secretory epithelium. Normal Golgi body height ranges from 6–12 μ. Only in lightly osmicated cells can one see the coiled structure of the knot. In the tissues of animals castrated for 6 days or more the normal large Golgi body is not evident but low knots of 4 μ, or more commonly, only osmicated clumps of granules are found. In the clear cells of the castrated animals these low clumps give a very specific indication of castration. The long time castrate effect is practically complete by 30 days following castration.

DISCUSSION

In the adult normal animal a tendency of conformity to a specific cell height range was demonstrated in all the tissues studied. Any extreme variation from the average is undoubtedly due to variations in physiological condition of the gland at the time of autopsy. The physiological condition is influenced by the amount of secretion accumulated in the tubule and the length of time since the last emptying of the gland by ejaculation. Warnock ('23) and Armisted ('25) describe the nature of the epithelium of the seminal vesicle

in the exhausted and the resting state. The average cell heights and the typical Golgi body recorded in the paper correspond to the resting cell picture.

It has been demonstrated by measurements that following castration the epithelial cells of the seminal vesicles, middle and posterior prostate and the vas deferens of the adult become reduced in height. This rate of degree of reduction in cell height is not uniform in the four kinds of tissue (fig. 2), but for each gland a distinct regression pattern is followed. This variable in regression pattern suggests differentials in testicular hormone threshold for each of the glands.

Animals of diverse genetic constitutions appear to show a considerable degree of variability in their responses to castration. With such a stock it appears that results can only be averaged from the study of several animals at each step in the experimental procedure. In this synthesis, when possible, at least five animals were examined for each comparable stage. In using the histology and cytology of the adult male guinea pig accessory organs as criteria for testicular hormone effects it appears that genetic make-up, age, weight, and physiological state, should be uniform to reduce the variability of results.

It is to be noted that except for the Golgi body no cell organelles such as granules, halos, light areas, etc., which could be used as qualitative indicators, were found in any of the glands. Fixatives and stains similar to those used by Moore, Price and Gallagher ('30) and Moore, Hughes and Gallagher ('30) on the rat were used in the preliminary work on the guinea pig but without evidence of these qualitative indicators.

One of the most interesting and yet most perplexing phases of this study has been the failure of response to castration on the part of the anterior prostate epithelium, both in the immature and adult animals. Even in the adult long time castrate the cell heights have remained practically unchanged. Golgi body impregnation has not been satisfactorily accomplished in normal or castrate tissues. Except for the more abundant

sloughing of cells into the lumen of the castrates it is difficult to distinguish histologically between the normal and the experimental animal. This may be partly explained by the work of Gley and Pézard ('21), who found that there was a gradual reduction in the secretion of the prostates of the castrated guinea pig. They further mention that prostate fluid from castrated animals was capable of coagulating the seminal vesicle secretion of normal animals. Walker ('10) has shown that this coagulating capacity is specific for the coagulating gland (anterior prostate). These facts and the results of this study allow one to question whether or not testicular hormone is necessary for a limited secretion of the matured anterior prostate gland. Certainly the normal amount of secretion is stopped following castration since the mass of the gland regresses 50% (table 1) within 15 days following castration.

SUMMARY

1. Accessory reproductive glands of the adult male guinea pig tend to increase in weight as the animal grows heavier, at least until a body weight of over 600 gm. is attained. Castration produces a reduction in accessory gland weights equal to 50% of the original.

2. Lumen diameters of acini and tubules are reduced by approximately one-third following castration.

3. Castration results in epithelial cell height reduction in all accessory glands with the exception of the anterior prostate.

4. Golgi bodies, demonstrable in all but the epithelium of the anterior prostate, are definitely altered and reduced following castration.

5. The above castration effects in the male guinea pig are either very pronounced or complete by 30 days following castration.

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